Rat Anti-Type I and Type II Collagen IgG Antibody ELISA Kits with TMB

Catalog # 1021T - 1025T, 2041T - 2045T

For Research Use Only - Not Human or Therapeutic Use

PRODUCT SPECIFICATIONS

DESCRIPTION:	Assay kit to quantify rat anti-collagen antibodies
FORMAT:	Pre-coated 96-well ELISA Plate with removeable strips
ASSAY TYPE:	Indirect ELISA
ASSAY TIME:	4.25 hours
STANDARD RANGE:	16 units/ml to 0.25 units/ml
NUMBER OF SAMPLES:	Up to 39 (duplicate) samples/standard plate (will vary for custom kits)
SAMPLE TYPES:	Serum and Plasma
RECOMMENDED SAMPLE DILUTIONS:	1:1000 (at least)
CHROMOGEN:	TMB (read at 450 nm)
STORAGE:	-20°C
VALIDATION DATA:	N/A
NOTES:	

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16928 Woodinville-Redmond Rd NE Suite B-101
Woodinville, WA 98072
Phone: 425.702.6365 or 888.246.6373
Fax: 425.882.3094
www.chondrex.com
support@chondrex.com

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INTRODUCTION

Heterologous type II collagen is widely used as an immunogen for the collagen-induced arthritis (CIA) model. In CIA-susceptible rat, the serum antibody levels to the type II collagen used for immunization are very high. Furthermore, these antibodies cross-react to various species of type II collagen including autologous type II collagen, due to the conserved amino acid sequences of type II collagen. It is important to note that although type I collagen shares about 80% of its amino acid sequence with type II collagen, it is not capable of inducing autoimmune-mediated diseases. This indicates that epitope specificity of antibodies and T-cells are important for establishing autoimmunity and subsequent development of autoimmune diseases. Therefore, type I collagen might be useful as a control for studying the pathogenesis of autoimmune mediated arthritis and B and T-cell epitope specificity.

This kit is designed to assay type I and type II collagen antibodies in rat serum. Chondrex Inc.'s ELISA systems incorporate unique blocking agents to reduce non-specific reactions. These agents reduce the background levels by inhibiting the hydrophobic binding of immunoreactive serum components in sample specimens onto plastic surfaces. Chondrex, Inc. offers various species of type I and type II collagen-coated strips as shown below.

KIT COMPONENTS

Item	Quantity	Amount	Storage
Standard Antibody (2046)	1 vial	20 units, lyophilized	-20°C
Secondary Antibody (Peroxidase-Conjugated Goat Anti-Rat IgG) (10214)	2 vials	50 µl	-20°C
Solution A - Blocking Buffer (2071)	1 bottle	12 ml	-20°C
Solution B - Sample/Standard Dilution Buffer (2072)	1 bottle	50 ml	-20°C
Solution C - Secondary Antibody Dilution Buffer (2073)	1 bottle	20 ml	-20°C
TMB (90023)	2 vials	0.2 ml	-20°C
Chromogen Dilution Buffer (90022)	1 bottle	20 ml	-20°C
Stop Solution - 2N Sulfuric Acid (9016)	1 bottle	10 ml	-20°C
Wash Buffer, 20X (9005)	1 bottle	50 ml	-20°C
Type I or Type II Collagen-Coated 8-Well Strips	10 each	8-well strips	-20°C
Reference Standard Strips (two strips per run)	4 each	8-well strips	-20°C

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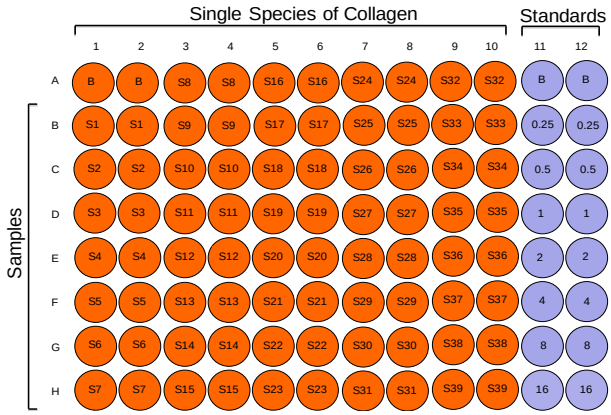
PLATE COATING AND SETUP

Species	Type I Collagen Color Coding – Catalog #	Type II Collagen Color Coding – Catalog #
Chick	(CI) Gold – 1021T	(CII) Yellow – 2041T
Bovine	(BI) Dark Blue – 1022T	(BII) Green – 2042T
Porcine	(PI) Brown – 1023T	(PII) Pink – 2043T
Rat	(RI) Lime Green – 1024T	(RII) Purple – 2044T
Human	(HI) Silver – 1025T	(HII) Blue – 2045T
Standard	Red	Red

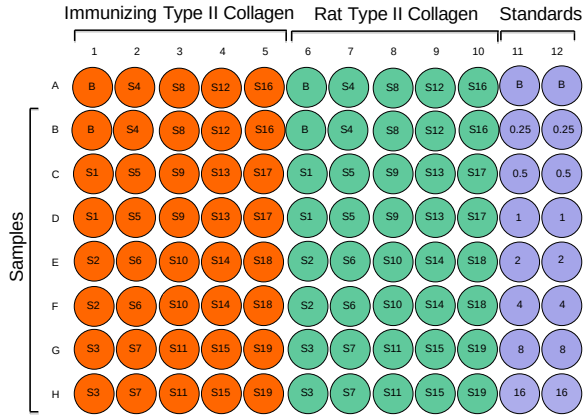
Standard ELISA Kit with One Species of Type I or Type II Collagen

A standard ELISA kit consisting of ten 8-well strips coated with one species of type II collagen and two 8-well strips for reference standards. “B” represents blank wells to determine background values caused by the secondary antibody. Standards and samples (1-39) are run in duplicate.



Custom ELISA Kit with Multiple Species of Collagen

A custom kit for assaying antibody levels to the immunizing type II collagen and their cross-reactivity to homologous rat type II collagen. “B” represents blank wells to determine background values caused by the secondary antibody. Standards and samples (1-19) are run in duplicate.



NOTES BEFORE USING ASSAY

NOTE 1: It is recommended that the standard and samples be run in duplicate.

NOTE 2: Warm up all buffers to room temperature before use.

NOTE 3: Crystals may form in Wash Buffer, 20X when stored at cold temperatures. If crystals have formed, warm the wash buffer by placing the bottle in warm water until crystals are completely dissolved.

NOTE 4: Measure exact volume of buffers using a serological pipet, as extra buffer is provided.

NOTE 5: Cover the plate with plastic wrap or a plate sealer after each step to prevent evaporation from the outside wells of the plate.

NOTE 6: For partial reagent use, please see the assay protocol’s corresponding step for the appropriate dilution ratio. For example, if the protocol dilutes 50 µl of a stock solution in 10 ml of buffer for 12 strips, then for 6 strips, dilute 25 µl of the stock solution in 5 ml of buffer. Partially used stock reagents may be kept in their original vials and stored at -20ºC for use in a future assay.

NOTE 7: This kit contains animal components from non-infectious animals and should be treated as potential biohazards in use and for disposal.

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ASSAY OUTLINE

Add 100 µl of blocking buffer into wells

↓ Incubate at room temperature for 1 hour.
Wash plate.

Add 100 µl of diluted standards and samples into wells

↓ Incubate at room temperature for 2 hours.
Wash plate.

Add 100 µl of diluted secondary antibody solution into wells

↓ Incubate at room temperature for 1 hour.
Wash plate.

Add 100 µl of TMB solution into wells

↓ Incubate at room temperature for 15 minutes.

Add 50 µl of Stop Solution into wells

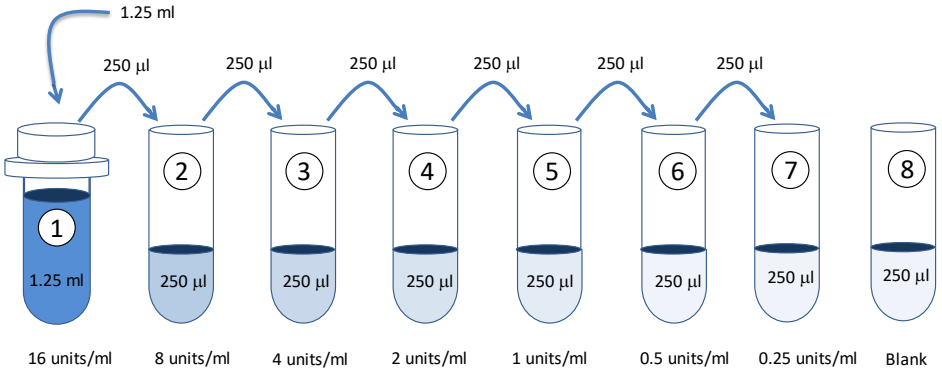
↓

Read plates at 450 nm/630 nm

ASSAY PROCEDURE

1. **Add Blocking Buffer**: Add 100 µl of Blocking Buffer (Solution A) to all wells. Incubate for 1 hour at room temperature.

2. **Prepare Standard Dilutions**: Dissolve one vial of standard (20 units/vial) in 1.25 ml of Sample/Standard Dilution Buffer (Solution B) to make a 16 units/ml solution. Prepare serial dilutions of the standard by mixing 250 µl of the 16 units/ml standard with 250 µl of Solution B - 8 units/ml. Then repeat this procedure to make five more serial dilutions of standard: 4, 2, 1, 0.5 and 0.25 units/ml solutions. The 16 units/ml standard may be stored at –20°C for use in a second assay. Chondrex, Inc. recommends making fresh serial dilutions for each assay.



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3. **Prepare Sample Dilutions**: Centrifuge serum samples at 10,000 rpm at room temperature for 3 minutes to remove insoluble materials and lipids. Dilute samples 1:1000 or more with Solution B. For example, dilute 10 µl of sample with 0.99 ml of Solution B (1:100). Keep this as a stock solution for future assays. If it is necessary to assay antibodies at a low dilution (less than 1:200) due to low antibody levels, please contact Chondrex, Inc. customer service at support@chondrex.com. In the CIA model, anti-collagen antibody levels may vary from zero (naïve rodents) to 1,000,000 units/ml or higher (arthritic rodents) depending on the immune response, arthritis severity, and time of sample collection. With unknown anti-collagen antibody levels, Chondrex, Inc. recommends testing several dilutions, such as 1:1000, 1:10,000, and 1:100,000, in order to find the appropriate dilution for the samples.

4. **Dilute Wash Buffer**: Dilute 50 ml of 20X wash buffer in 950 ml of distilled water (1X wash buffer). Wash the plate with 1X wash buffer at least 3 times using a wash bottle with manifold or an automated plate washer. Empty the plate by inverting it and blotting on a paper towel to remove excess liquid. *Do not allow the plate to dry out.*

5. **Add Standards and Samples:** Add 100 µl of standards, Solution B (blank) and samples to wells in duplicate. Incubate at room temperature for 2 hours.

6. **Wash:** Wash the plate with 1X wash buffer at least 3 times using a wash bottle with manifold or an automated plate washer. Empty the plate by inverting it and blotting on a paper towel to remove excess liquid. *Do not allow the plate to dry out.*

7. **Add Secondary Antibody:** Dilute one vial of secondary antibody in 10 ml Secondary Antibody Dilution Buffer (Solution C). Add 100 µl of secondary antibody solution to each well and incubate at room temperature for 1 hour.

Strip #	Secondary Antibody (µl)	Solution C (ml)
2	8	1.7
4	17	3.3
6	25	5.0
8	33	6.6
10	42	8.2
12	50	10.0

8. **Wash:** Wash the plate with 1X wash buffer at least 3 times using a wash bottle with manifold or an automated plate washer. Empty the plate by inverting it and blotting on a paper towel to remove excess liquid. *Do not allow the plate to dry out.*

9. **TMB:** Use new tubes when preparing TMB. Dilute one vial (200 µl) of TMB in 10 ml of Chromogen Dilution Buffer just prior to use. Add 100 µl of TMB solution to each well immediately after washing the plate. Incubate for 15 minutes at room temperature.

Strip #	TMB (µl)	Chromogen Dilution Buffer (ml)
2	34	1.7
4	66	3.3
6	100	5.0
8	132	6.6
10	164	8.2
12	200	10.0

10. **Stop:** Add 50 µl of 2N sulfuric acid (Stop Solution) to each well.

11. **Read Plate:** Read the OD values at 450 nm. If the OD values of samples are greater than the OD values of the highest standard, re-assay the samples at a higher dilution. A 630 nm filter can be used as a reference.

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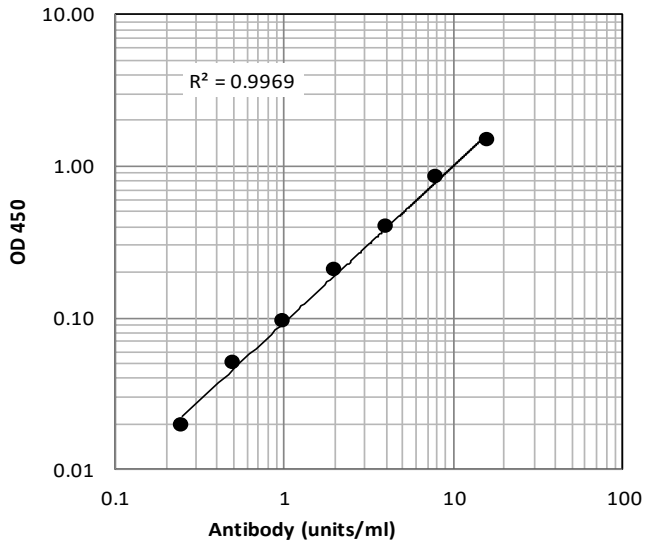
CALCULATION OF ANTIBODY TITERS

1. Average the duplicate OD values for the standards, blanks (B) and test samples.
2. Subtract the blank (B) values from the averaged OD values of the standards and test samples.

   NOTE: Individual antigens have unique background values. Therefore, blank wells should be used for each different antigen.
3. Plot the OD values of standards against the units/ml of antibody standard. Using a log/log plot will linearize the data. Figure 1 shows a representative experiment where the standard range is from 0.25 to 16 units/ml.
4. The units/ml of antibody in test samples can be calculated using regression analysis.

   NOTE: 82 units is approximately 1 µg IgG antibody/ml.

Figure 1 - A Typical Standard Curve for the Rat Anti-Type I and Type II Collagen IgG Antibody ELISA Kit with TMB.



TROUBLESHOOTING

For frequently asked questions about assays and ELISAs, please see Chondrex, Inc.'s ELISA FAQ for more information.

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16928 Woodinville-Redmond Rd NE Suite B-101
Woodinville, WA 98072
Phone: 425.702.6365 or 888.246.6373
Fax: 425.882.3094
www.chondrex.com
support@chondrex.com